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厦门大学

硕士学位论文

海洋原甲藻（*Prorocentrum micans*）对不同生态习性桡足类存活、发育的影响及蛋白质组学分析

Effects of *Prorocentrum micans* on survival and development of different ecological habits copepods and proteomics analysis

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缩略词表

缩略词	英文全称	中文全称
2-DE	Two dimensional electrophoresis	双向电泳
A/B	Acrylamide/Bis solution	丙烯酰胺溶液
APS	Ammonium persulfate	过硫酸铵
BSA	Bovine serum albumin	牛血清蛋白
IAA	Iodoacetamide	碘乙酰胺
DTT	Dithiothreitol	二硫苏糖醇
IEF	Isoelectric focuse	等电聚焦
EDTA	Ethylenediaminetetraacetic acid	乙二胺四乙酸
IPG	Immobilized pH gradients	固相胶条
MALDI-TOF/TOF	Matrix assisted laser desorption/ionization time-of-flight mass spectrometry	飞行质谱-质谱联用
PAGE	Polyacrylamide gel electrophoresis	聚丙烯酰氨凝胶电泳
PBS	Phosphate buffered saline	磷酸缓冲液
RB	Rehydration buffer	溶胀液
SDS	Sodium dodecylsulfate	十二烷基磺酸钠
TCA	Trichloroacetic acid	三氯乙酸
TEMED	N,n,n’,n’-tetramethylethylenediamine	四甲基乙二胺
TGS	Tris-glycine SDS	电泳缓冲液
Tris-HCl	Tris(hydroxymethyl) aminomethane hydrochloride	三羟甲基甲烷盐酸盐
PI	Isoelectric point	等电点
MW	Molecular weight	分子量
Hsp	Heat shock protein	热休克蛋白
GAPDH	glyceraldehyde-3-phosphate dehydrogenase	甘油醛-3-磷酸脱氢酶

摘 要

本文运用实验生态方法研究了海洋原甲藻（*Prorocentrum micans*）对 3 种不同生态习性桡足类[日本虎斑猛水蚤（*Tigriopus japonicus*）、婆罗异剑水蚤（*Apocyclops borneoensis*）和太平洋纺锤水蚤（*Acartia pacifica*）]不同发育阶段的存活率和发育时间的影响，通过太平洋纺锤水蚤五个世代的连续培养，分析了多世代培养条件下太平洋纺锤水蚤对不同浓度海洋原甲藻的适应能力；同时，采用蛋白质组学及生物信息学方法，对不同浓度海洋原甲藻培养条件下的太平洋纺锤水蚤和日本虎斑猛水蚤、多世代培养的太平洋纺锤水蚤进行了差异蛋白表达分析和质谱分析，探究海洋原甲藻对不同生态习性桡足类的影响机制。主要结果如下：

1. 不同生态习性的桡足类在各发育阶段的存活率因海洋原甲藻浓度的不同而具有显著差异（$P<0.05$）。在同一饵料浓度下，3 种桡足类的存活率基本为：日本虎斑猛水蚤>婆罗异剑水蚤>太平洋纺锤水蚤。尤其在低饵料浓度下（0.07 μgC mL^{-1} 和 0.35 μgC mL^{-1}），3 种桡足类存活率差异特别显著（$P<0.05$），太平洋纺锤水蚤最低（整个世代发育阶段的存活率分别为 4%和 18.67%），日本虎斑猛水蚤最高（整个世代发育阶段的存活率分别为 51.7%和 62%）。就发育时间而言，3 种桡足类各阶段发育时间不尽相同。无节幼体阶段，随饵料浓度的升高，太平洋纺锤水蚤发育时间极显著缩短（0.07 μgC mL^{-1} 和 8.50 μgC mL^{-1} 浓度下发育时间分别为 230.33 h 和 103.67 h），婆罗异剑水蚤发育时间随饵料浓度的升高也有较显著的缩短（0.07 μgC mL^{-1} 和 8.50 μgC mL^{-1} 浓度下发育时间分别为 90.2 h 和 65.9 h），日本虎斑猛水蚤的发育时间并无显著变化（0.07 μgC mL^{-1} 和 8.50 μgC mL^{-1} 浓度下发育时间分别为 103.2 h 和 98.5 h）。

2. 在连续世代培养条件下，太平洋纺锤水蚤无节幼体的存活率因海洋原甲藻浓度的不同而具有显著差异（$P<0.05$）。较高浓度下，太平洋纺锤水蚤无节幼体存活率随着世代数增加而升高。在 5 个连续世代的培养中，太平洋纺锤水蚤各发育阶段的发育时间因投喂海洋原甲藻浓度的不同也均有显著差异（$P<0.05$）。从无节幼体阶段的发育时间来看，投喂低浓度海洋原甲藻（0.07 μgC mL^{-1}，0.35 μgC mL^{-1}）时，太平洋纺锤水蚤无节幼体的发育时间显著多于投喂高浓度饵料的（1.70

μgC mL^{-1}，5.10 μgC mL^{-1}，8.50 μgC mL^{-1}）无节幼体（$P<0.05$）。

将太平洋纺锤水蚤各世代无节幼体的存活率、发育时间随饵料浓度的变化趋势进行二项式拟合，可以发现，经过五个世代的连续培养，太平洋纺锤水蚤的最适饵料浓度逐渐升高，表现出一定的遗传适应。在多世代培养过程中，同一饵料浓度下，无节幼体阶段的太平洋纺锤水蚤在存活率和发育时间的变化上都最显著，最适饵料浓度的变化也最为显著，呈现出明显的升高趋势，由此可知，太平洋纺锤水蚤无节幼体期是对饵料浓度变化最为敏感的阶段。

3. 双向电泳分离和质谱鉴定结果表明，随着海洋原甲藻浓度的升高，太平洋纺锤水蚤的 3 个蛋白点表达量降低，2 个蛋白点表达量升高，另有 1 个蛋白点仅在高浓度下表达。在质谱鉴定出的蛋白中，谷氨酸脱氢酶、甘油醛-3-磷酸脱氢酶与能量代谢有关，热休克蛋白 70 与肌动蛋白可增强细胞蛋白稳定性，达到保护细胞作用。而日本虎斑猛水蚤随着海洋原甲藻浓度的升高，2 个蛋白点表达上调，6 个蛋白点表达下调，另有 1 个蛋白点不表达。在质谱鉴定出的蛋白中精氨酸激酶与能量代谢有关，肌动蛋白起到维持细胞蛋白稳定，促进机体正常生命活动的作用。

4. 相同饵料浓度条件下，不同生态习性桡足类的全蛋白表达具有显著差异，这主要与其响应外界环境的身体机制不同有关。研究发现，日本虎斑猛水蚤体内 4 种蛋白的表达显著高于太平洋纺锤水蚤。同时，精氨酸激酶、卵黄原蛋白、肌动蛋白、苹果酸酶、丙酮酸脱氢酶 E1-β 亚基、原肌球蛋白、磷酸丙糖异构酶、膜黏连蛋白 2 等 10 种蛋白仅发现在日本虎斑猛水蚤体内表达，这些蛋白都广泛参与了机体内的能量代谢、生殖发育过程中。

5. 经过连续世代培养，第一世代太平洋纺锤水蚤与第五世代太平洋纺锤水蚤相比发现了 2 种差异蛋白，即 ATP 合成酶 β 亚基和原肌球蛋白。这 2 种蛋白与桡足类体内的能量代谢和物质运输紧密相关，其表达的上调或下降都将直接影响正常的新陈代谢活动。因此相同饵料条件下，经连续世代培养后，太平洋纺锤水蚤的存活率和发育时间都会表现出显著的变化。

本文采用实验生态与分子生物学相结合的方法，分析了不同生态习性桡足类对海洋原甲藻浓度变化的响应，同时利用现代分子信息学技术，探究了多世代培

养下的桡足类对环境的适应能力，对于深入探讨浮游微藻和桡足类之间的生态关系及其影响机制具有重要的理论意义。

关键词：海洋原甲藻；日本虎斑猛水蚤；婆罗异剑水蚤；太平洋纺锤水蚤；存活；蛋白质组学；质谱分析

ABSTRACT

The effects of *Prorocentrum micans* on the survival and development of *Tigriopus japonicas*, *Apocyclops borneoensis* and *Acartia pacifica* were studied by experimental ecology research methods. Moreover, the ecophysiological responses of three different ecological hatits of copepods to *P. micans* were discussed. Furthermore, *A. pacifica* were fed with *P. micans* at different concentrations in five consecutive generation so that investigating effects of those treatments on *A. pacifica.* Meanwhile, techniques of proteomic and bio-information were used to study the protein different expressions in *T. japonicus* and *A. pacifica* when fed with different concentrations of *P. micans.* Also, analysis on the mechanism of different proteins in bio-metabolism aims at studying the different protein expression in biological metabolism. The results were as followed:

1. At different development stages, significant differences in survival were caused by fed with different concentrations of *P. micans* in three different ecological hatits of copepods ($P<0.05$). The effects of the same concentration of *P. micans* on survival of three copepods was significant, especially at the low concentration of *P. micans* ($P<0.05$). At low concentrations of *P. micans* (0.07 μgC mL^{-1} and 0.35 μgC mL^{-1}), the survival of *T. japonicas* was highest (51.7% and 62%), followed by *A. borneoensis* and *A. pacifica* (4% and 18.67%). In naupliar development, the duration of development phases of three copepods was different when fed with different concentrations of *P. micans*. With the increase of feeding concentration from the lowest level of 0.07 μg C mL^{-1}, developmental time of nauplii decreased significantly for the *A. pacifica* ($P<0.01$) (230.33 h and 103.67 h respectively) as well as *A. borneoensis* (90.2 h and 65.9 h respectively) in the 0.07 μgC mL^{-1} and 8.50 μgC mL^{-1} concentrations of *P. micans*. However, there was no significant variation in *T. japonicas* and the developmental time were 103.2 h and 98.5 h.

2. In five consecutive generations, the survival of the naupliar stage (N_I- N_{VI}) of *A. pacifica* was significantly affected by different concentrations for the *P. micans* ($P<0.05$). It found that survival increased with variation of generations at high concentrations of *P. micans.* The duration of the different development phase was significantly different when fed with different concentrations for the algae ($P<0.05$). With the increase of feeding concentration from the low concentraions of *P. micans*

(0.07 μg C mL^{-1} and 0.35 μg C mL^{-1}), developmental time of nauplii decreased significantly ($P<0.05$).

With the binomial fitting of survival and development time of *A. pacifica*, it found that the optimal feeding concentration was tend to be higher. This might show that *A. pacifica* has genetically adapted to cope with high concentration of the algae. The naupliar stage (N_I- N_{VI}) of *A. pacifica* was the most sensitive phase because of the most apparent variation of survival and development time in different generations.

3. The protein different expressions in *A. pacifica* when fed with different concentrations of *P. micans* were analysized. With the increase of feeding concentration, three protein spots were down-regulated while two proteins spots were up-regulated and one protein was expressed at high concentration. In the identified proteins, glutamate dehydrogenase and glyceraldehyde-3-phosphate dehydrogenase showed positive correlation with energy metabolism. Heat shock protein 70 and action can improve the stability of cellular structure. Furthermore, the protein different expressions in *T. japonicus* when fed with different concentrations of *P. micans* were analysized. With the increase of feeding concentration, there were six protein spots are down-regulated while two proteins spots are up-regulated and one protein are expressed at low concentration. In the identified proteins, arginine kinase associated with energy metabolism and actin can keep protein stability.

4. The protein different expressions in different ecological hatits copepods when fed same concentration of *P. micans* were analyzed. There were apparent difference in protein expressed between them. Ten proteins were only expressed and four protein spots were up-related in *T. japonicas.* These proteins were mitochondrial deoxynucleotide carrier, arginine kinase, vitellogenin, actin, malate dehydrogenase, pyruvate dehydrogenase E1 component subunit beta, triosephosphate isomerase and annexin 2. They were widely involved in energy metabolism and reproductive development.

5. The protein different expressions in different between first generation and fifth generation of *A. pacifica* were analysized. Two proteins were identified, namely, ATP synthase subunit beta and tropomyosin which affect the normal life activities that result in the differences in survival and development of copepods.

In our research, experimental ecology research methods and techniques of

proteomic and bio-information were combined to investigate the response of different ecological hatits copepods to different concentrations of *P. micans*. Proteomics methods were firstly used to study different protein expression of copepods in different generations which was worthwhile for research relations between planktonic algae and copepods.

Key Words: *Prorocentrum micans*; *Tigriopus japonicas*; *Apocyclops borneoensis*; *Acartia pacifica*; Survival; Proteomics; MALDI-TOF-TOF-MS.